

Atomic Absorption

Author

Praveen Sarojam, Ph.D.

PerkinElmer, Inc.
Shelton, CT 06484 USA

Quantification of Essential Metals in Spice Mixtures for Regulatory Compliance Using Flame Atomic Absorption Spectrophotometry

Introduction

Foods, together with water, provide the major proportion of the total daily intake of trace elements by humans. Spices and vegetables are some of the most common foods in the human diet around the world. Besides polluted soil and water, foods can also be contaminated with trace metals by the introduction of mechanized farming, the increasing use of chemicals, food processing and packaging, etc. In order to minimize adverse impact, it is important to measure and continuously monitor the levels of trace elements in various kinds of food materials. Trace element food composition data are also important for both consumers and health professionals. In recent years, food labeling legislation has enforced this requirement. Trace element determination in complex matrices, such as food, often requires sample preparation prior to determination by instrumental techniques.¹

Cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) are all essential elements, not only for mammals, but also for plants. They play important roles in many biological processes including carbohydrate and lipid metabolism.² For example, a daily copper intake of 1.5 - 2.0 mg is essential and copper at nearly 40 ng/mL is required for normal metabolism of many living organisms.³ However, copper at higher levels is toxic to the circulatory system and kidneys. The trace element content of food items for all the essential elements mentioned above must be controlled on a daily basis.

There is an increasing need to monitor the essential element levels in food samples at ever decreasing concentrations. For this purpose, very sensitive, yet rapid and inexpensive methods are necessary. The quantification of trace metals in food samples has routinely been carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES),⁴ inductively coupled plasma mass spectrometry (ICP-MS),^{5,6} graphite furnace atomic absorption spectrophotometry (GFAAS)⁷ and flame atomic absorption spectrophotometry (FAAS).^{8,9,10} Compared with other techniques, FAAS has the characteristics of good precision and simplicity with lower cost and minimum operator proficiency. The objective of this work is two-fold: (1) to accurately analyze, using FAAS, the levels of essential metals (in particular: cobalt, copper, manganese, nickel and zinc) that may be present in some major spice brands available on the market; (2) to cross-reference the measured levels to the recommended maximum tolerable daily intake limits specified by the U.S. Department of Agriculture (USDA).

Experimental Conditions

Instrumentation

The measurements were performed using a PerkinElmer® PinAAcle™ 900T atomic absorption spectrophotometer (Shelton, CT, USA) equipped with the intuitive WinLab32™ for AA software, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance. The high-efficiency optical system and solid-state detector used in this spectrophotometer provide outstanding signal-to-noise ratios. The solid-state detector is also highly efficient at low UV and longer wavelengths at the same time. The instrumental conditions for flame experiments are given in Table 1 (Page 3). A high-sensitivity nebulizer (Part No. N3160144) was used and the read time was 3.0 seconds for all samples. The signal type was AA and the calibration equation was linear through zero.



Figure 1. PerkinElmer PinAAcle 900T atomic absorption spectrophotometer.

A microwave sample preparation system was used for the digestion of spice samples and the certified reference material (CRM). This is an industrial-type microwave oven which is equipped with various accessories to optimize the sample digestion. The samples were digested using 100 mL high-pressure vessels made of PTFE. The sample digestion was performed in accordance with the program given in Table 2 (Page 3), as per U.S. Environmental Protection Agency (EPA) Method 3052.

Standards, Samples and Certified Reference Material Preparation

Single-element PerkinElmer Pure Calibration Standards for atomic spectroscopy were used as the stock standards for preparing the working standards (Part Nos. Co: N9303766; Cu: N9300183; Mn: N9303783; Ni: N9300177; Zn: N9300178). Working standards were prepared by serial volume/volume (v/v) dilution in 50 mL conical free-standing polypropylene vials (Part No. B0193234). Four-point calibration curves (three standards and one blank) were constructed for each individual metal ion and their calibration curve correlation coefficients (r^2) were better than 0.999 before the start of the sample analysis (Appendix I – Page 5).

ASTM® Type I water (Millipore® Corporation, Billerica, Massachusetts, U.S.) acidified with 0.2% nitric acid (Tampure®, TAMA Chemicals, Japan) was used as the calibration blank and for all dilutions. NIST® 1568a CRM for Trace Metals in Rice Flour was used to validate the method. Quality control (QC) check standards were prepared at the calibration curve midpoint for each individual element. Three branded powdered spice and herb samples available in India (coriander powder, ginger powder, and black pepper powder) were bought from a supermarket to be analyzed.

Approximately 0.5 g of each sample or CRM was accurately weighed in duplicate, then transferred to a digestion vessel. The digested samples were brought up to 25 mL in polypropylene vials with 0.2% HNO_3 . All sample vessels were cleaned by soaking with 10% v/v HNO_3 for at least 24 hours and rinsed abundantly in de-ionized water before use.

Table 1. Optimized experimental conditions of PinAAcle 900T.

Analyte	Co	Cu	Mn	Ni	Zn
Wavelength (nm)	240.73	324.75	279.48	232.00	213.86
Slit (nm)	0.2	0.7	0.2	0.2	0.7
Lamp Current (mA)	30	15	20	25	15
Calibration Stds (mg/L)	0.25, 0.5, 1.0	0.16, 0.32, 0.64	0.125, 0.5, 1.0	0.5, 1.0, 2.0	0.06, 0.12, 0.24
r ²	0.9995	0.9999	0.9998	0.9994	0.9999
QC Std (mg/L)	0.50	0.32	0.50	1.0	0.12
Lamp Type	HCL	HCL	HCL	HCL	HCL
Lamp Part No.	N3050118	N3050121	N3050152	N3050145	N3050191

Table 2. Program used for the digestion of spices and herbs.

Sequence	1	2
Power (watts)	1000	0
Ramp Time (min)	10	0
Hold Time (min)	10	20
Weight Taken (mg)	~500	
H ₂ O ₂ (mL)	1.0	
HNO ₃ (mL)	7.0	
Temp (°C)	180	

Results and Discussions

The role of the sample introduction system is of paramount importance in optimizing the short-term stability of signals. Furthermore, the best sensitivity can be achieved by careful optimization of flame conditions. WinLab32™ for AA software comes with a unique “Optimize Gas Flows” feature which helps to achieve the best possible sensitivity. This is an extremely important feature to assist in the analysis of low-level analytes in high matrix samples. The new easy-fit sample introduction system with the inert polymer spray chamber and high-precision nebulizer maximizes short-term stability and sensitivity even for the high acid matrix samples like the one used here. This is evident from the excellent percent relative standard deviation (%RSD) values obtained for low concentration standard signals (Table 3).

Table 3. Precision at midpoint of calibration for 10 replicate readings (%RSD).

Analyte	% RSD	Concentration (mg/L)
Co	0.8	0.50
Cu	0.7	0.32
Mn	0.9	0.50
Ni	0.7	1.0
Zn	0.9	0.12

The agreement between the certified values of the CRM and the measured values were good, demonstrating the accuracy of the generated calibration, as well as the overall accuracy of the developed method (Table 4). Method detection limits (MDLs) were calculated (Table 5) based on the standard deviation of seven replicates of the reagent blank (Student's t-value = 3.14, $\rho = 0.02$) and took into consideration the dilution factor of the samples. These limits were obtained under routine operating conditions. The extremely low detection limits obtained show the ability of the PinAAcle 900T spectrometer to analyze difficult matrices at the measured concentrations.

Table 4. Analysis of certified reference material by FAAS.

Analyte	NIST® 1568a Rice Flour	
	Certified Value (µg/g)	Measured Value (µg/g)
Cu	2.4 ± 0.3	2.30 ± 0.7
Mn	20.0 ± 1.6	20.0 ± 0.2
Zn	19.4 ± 0.5	18.8 ± 0.1

**Co and Ni were not reference elements.*

Table 5. Typical method detection limits (MDLs) for the analysis of essential elements in spices.

Analyte	MDL (mg/L)
Co	0.50
Cu	0.45
Mn	0.15
Ni	0.30
Zn	0.10

Post-digestion recovery studies were carried out and the results are summarized in Table 6. The recoveries obtained for post-digestion spikes easily met the U.S. EPA guideline of $\pm 15\%$. However, it is known that incomplete mineralization of samples during the microwave digestion process may cause difficulty in transferring analytes into solution and disturbances in the spectrochemical measurements.¹¹

The spice analysis results are summarized in Table 6. For example, the maximum tolerable daily intake limit specified by the USDA for copper is 10.0 mg. The results (Table 7) show that the level of Cu is not going to exceed the maximum tolerable daily intake limits specified by the USDA, assuming that no one would be consuming ≥ 300 g of the spice mixture on a daily basis.

Conclusions

Methods were developed for the accurate determination of Co, Cu, Mn, Ni, and Zn in spice mixtures using the PinAAcle 900T atomic absorption spectrometer in the FAAS mode and microwave digeston. The results confirmed that the determination of copper, manganese, cobalt, nickel and zinc in spices, after acid solubilization by microwave digestion, can be performed by FAAS on the PinAAcle 900T without any interferences. The PinAAcle 900H (Flame and Deuterium Furnace) and PinAAcle 900F (Flame only) spectrometers can also be used for this application.

References

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Table 6. Post-digestion spike recovery study – concentrations are based on the diluted solutions (two replicates (n=2) were performed for each sample).

Analyte	Sample Recovery (%)		Spike Level (mg/L)
	Black Pepper Powder	Ginger Powder	
Co	90	96	0.5
Cu	101	106	0.16
Mn	94	87	0.13
Ni	90	90	0.5
Zn	102	105	0.06

Table 7. Concentration of metals in spice and herb samples using FAAS compared to USDA guidelines (two replicates (n=2) were performed for each sample and sample duplicate).

Analyte	USDA Regulatory Level (mg)	Black Pepper Powder (mg/kg)		Coriander Powder (mg/kg)		Ginger Powder (mg/kg)	
		Sample	Duplicate	Sample	Duplicate	Sample	Duplicate
Co	*	0.80	0.82	0.65	0.75	1.5	1.3
Cu	10.0	32.3	33.3	13.9	13.8	5.9	5.6
Mn	11.0	185	189	145	145	770	745
Ni	*	4.1	4.0	1.7	1.4	2.3	2.1
Zn	40.0	39	36	74	71	50	51

*No USDA reference value for specified tolerable upper intake level was found.

Appendix I – Calibration Graphs for Different Analytes

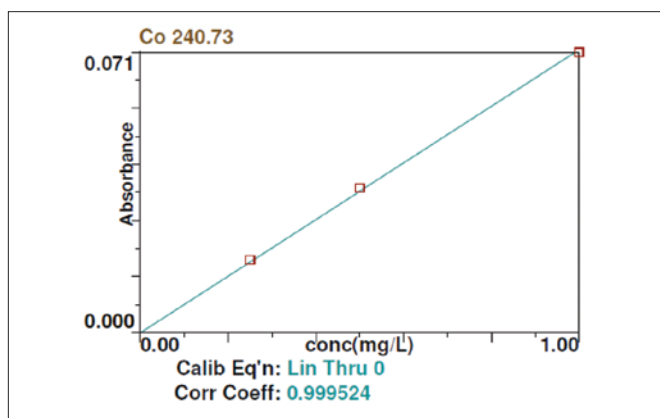


Figure 2. Calibration curve for the detection of Co using FAAS.

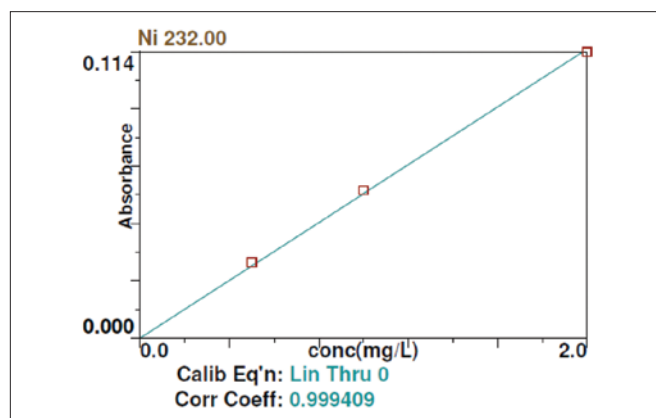


Figure 5. Calibration curve for the detection of Ni using FAAS.

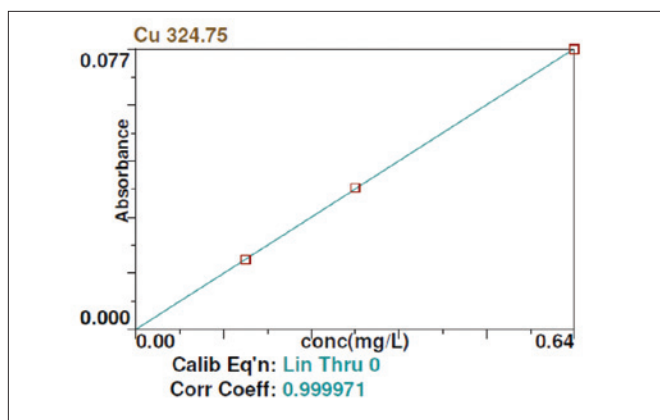


Figure 3. Calibration curve for the detection of Cu using FAAS.

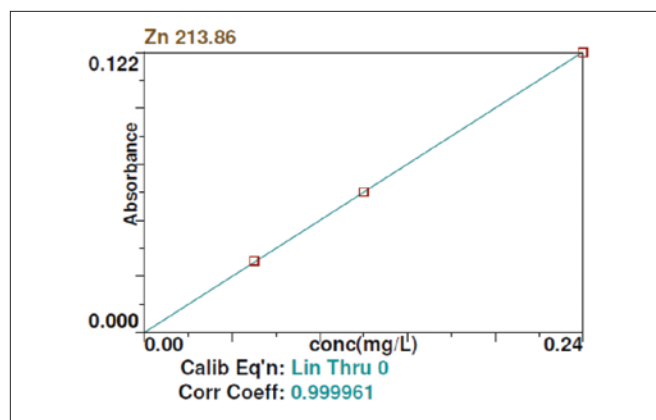


Figure 6. Calibration curve for the detection of Zn using FAAS.

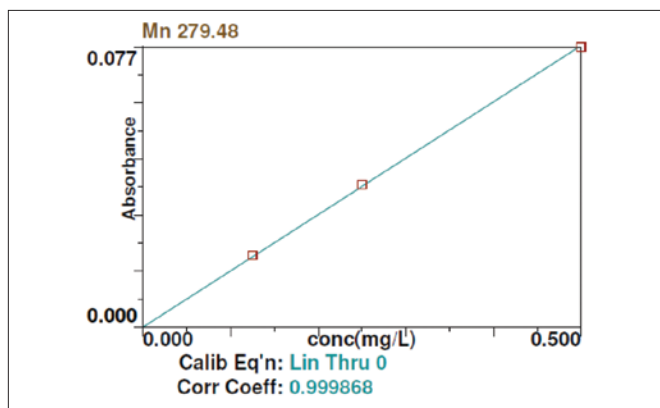


Figure 4. Calibration curve for the detection of Mn using FAAS.